Aphid (Hemiptera: Aphididae) Resistance in Wheat Near-Isogenic Lines

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J. Econ. Entomol. 97(2): 646–653 (2004)

ABSTRACT Plant and aphid biomass, photosynthetic pigment (chlorophylls *a* and *b* and carotenoids) concentrations, and chlorophyll *a*/*b* and chlorophyll/carotenoid ratios were quantified in aphid-infested 'Tugela' near-isogenic lines (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*). The objectives were to quantify changes of photosynthetic pigments (chlorophylls *a* and *b*, and carotenoids) caused by aphid feeding and assess resistance of wheat isolines through aphid and plant biomass analysis. Biomass of bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae)-infested plants was lower than Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae),-infested plants. When infested by *D. noxia*, all lines showed increased biomass over time, except Tugela where biomass decreased on day 12. No difference in plant biomass was detected among *R. padi*-infested and uninfested wheat lines. Biomass of *D. noxia* from Tugela (*D. noxia*-susceptible) was significantly higher than from plants with *Diuraphis noxia*-resistant *Dn* genes. *Diuraphis noxia* biomass from Tugela-*Dn1* and *Dn2* lines was not different from each other, but they were lower than from Tugela-*Dn5*. In contrast, there was no difference in *R. padi* biomass among wheat lines. Concentrations of chlorophylls *a* and *b* and carotenoids were significantly lower in *D. noxia*-infested plants compared with *R. padi*-infested and uninfested plants. When infested by *D. noxia*, chlorophyll *a* and *b* concentrations were not different among wheat lines on day 3, but they were lower in Tugela and Tugela-*Dn1* than in Tugela-*Dn2* and -*Dn5* plants on days 6 and 12. However, no difference was detected in chlorophyll *a*/*b* or chlorophyll/carotenoid ratio among Tugela lines. The study demonstrated that *Dn* genes in the Tugela isolines conferred resistance to *D. noxia* but not to *R. padi*. Tugela-*Dn1* was antibiotic, Tugela-*Dn2* was tolerant and antibiotic, and Tugela-*Dn5* was moderately antibiotic.

KEY WORDS *Diuraphis noxia*, *Rhopalosiphum padi*, chlorophylls *a* and *b*, carotenoids, chlorophyll *a*/*b* ratio

CHLOROPHYLL CATABOLISM CAN BE differentiated into two types. One is chlorophyll catabolism associated with normal progressive senescence in plants, whereas the other is leaf chlorosis in growing plants elicited by herbivore feeding, nutritional deficiencies, or pathogen infections (Ni et al. 2002). Although chlorophyll degradation in vitro has been well documented (Janave 1997, Matile et al. 1999, Dangl et al. 2000, Takamiya et al. 2000), the mechanism of chlorosis caused by herbivore infestation remains unclear.

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae), is an important pest that causes chlorosis on cereal crops. *Diuraphis noxia*-elicited chlorosis indicates the loss of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) that are vital for plant growth. Without them, leaves cannot absorb light and therefore cannot store energy. All photosynthetic organisms contain one or more organic pigments capable of absorbing visible radiation that will initiate the photochemical reactions of photosynthesis (Blankenship 2002). The major classes of pigments found in plants, bacteria, and algae are the chlorophylls (chlorophyll *a, b, c,* and *d*), bacteriochlorophylls (bacteriochlorophyll *a, b, c, d, e, f* and *g*), carotenoids (β-carotene, α-carotene, luteol, violaxanthol, and fucoxanthol), and phycobilins (i.e., phycoerythrins, phycocyanins, and allophycocyanins) (Hall and Rao 1992, Biswal 1995). The pigments in higher plants mainly consist of chlorophyll *a*, chlorophyll *b*, and most of the carotenoids (Blankenship 2002).

Chlorophylls *a* and *b* have an absorption maximum at 663 and 645 nm, respectively, in acetone that gives them the characteristic green color. Chlorophylls *a* and *b* act as primary light harvesters in plant photo-

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0022-0493/04/0646–0653$04.00/0 © 2004 Entomological Society of America

synthesis. Carotenoids, which are responsible for the orange-yellow colors observed in the leaves of plants, absorb light between 400 and 500 nm, a range in which absorption by chlorophylls is relatively weak. As such, carotenoids play a minor role as accessory light-harvesting pigments, absorbing and transferring light energy to chlorophyll molecules (Malkin and Niyogi 2000). Most importantly, carotenoids function in a process called photoprotection. Under the high light intensities often found in nature, plants may absorb more light energy than they can actually use for photosynthesis. This excessive excitation of chlorophylls can result in increased formation of the singlet oxygen that is detrimental to plant photosynthesis (Malkin and Niyogi 2000, Blankenship 2002). Carotenoids are able to accept excitation energy and prevent singlet oxygen formation (Malkin and Niyogi 2000, Blankenship 2002). Therefore, reduction of chlorophylls or carotenoids in plants induced by herbivore infestation may negatively affect the photosynthetic capacity of plants.

Many researchers have assessed the impact of sap-feeding herbivore-elicited photosynthetic pigment changes among resistant and susceptible cereal plants and their effects on plant photosynthetic efficiency (Kruger and Hewitt 1984, Riedell 1989, Burd and Todd 1992, Miller et al. 1994, van der Westhusizen and Pretorius 1995, Burd and Elliott 1996, Ni et al. 2002, Macedo et al. 2003, Heng-Moss et al. 2003). The objectives of this study involve using a series of wheat lines (Tugela and Tugela near-isogenic lines [isolines]) with different *D. noxia* resistance genes (*Dn1*, *Dn2*, and *Dn5*) to quantify changes of photosynthetic pigments (chlorophylls *a* and *b* and carotenoids) caused by aphid [*D. noxia*, and the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae)] feeding and assess wheat resistance through quantification of aphid and plant biomass. Correlation between photosynthetic pigment variations and *Dn* genes among Tugela isolines provided baseline information for the study of gene function in wheat.

Materials and Methods

Insects and Plants. *Diuraphis noxia*, a chlorosis-eliciting species, and *R. padi*, a nonchlorosis-eliciting species, were obtained from colonies established from field collections. The *D. noxia* colony was established from aphids collected near Scottsbluff, NE, in 1994, whereas the *R. padi* colony was established from aphids collected near Lincoln, NE, in 1996 (Ni et al. 2001). Aphids were maintained on 'Stephens' (*D. noxia*-susceptible) wheat in Plexiglas cages (30 by 15 by 15 cm) in separate Conviron growth chambers (Controlled Environments Ltd., Winnipeg, MB, Canada) at 21°C, with a photoperiod of 16:8 (L:D) h and 40–50% RH.

Wheat lines used in the experiment varied in their susceptibility to *D. noxia.* The 'Tugela' wheat was *D. noxia* susceptible, whereas the near-isogenic lines varied in *D. noxia* resistance (Tugela-*Dn1*, antibiosis; Tugela-*Dn2*, tolerance; and Tugela-*Dn5*, antixenosis and antibiosis). Seeds were planted at the rate of three plants per Conetainer (3.81 cm in diameter by 21 cm in depth) (Stuewe and Sons, Inc., Corvallis, OR). Conetainers were filled with Sunshine soil mix No. 1 (SunGro Horticulture, Bellevue, WA) and placed in Conetainer racks (61 by 30 by 18 cm), leaving a space among Conetainers to provide adequate light. Plants were watered uniformly from the bottom by placing a rack over a plastic tray (54 by 28 by 6 cm) filled with water. Before aphid infestation, plants were thinned to two seedlings per Conetainer. Experiments were maintained in a growth chamber at 21°C with a photoperiod of 16:8 (L:D) h and 40–50% RH.

Aphid Preconditioning and Infestation. Each aphid species was preconditioned on Stephens wheat caged with polyethylene tubes (30 cm in length by 4 cm in diameter) in a Conviron growth chambers at 21°C with a photoperiod of 16:8 (L:D) h and 40–50% RH (Schotzko and Smith 1991). Adults ($n = 10$) were placed on Stephens wheat plants at the three-leaf stage (Zadoks stage 13) (Zadoks et al. 1974) and removed after 3 d. Nymphs were maintained on the plants for ≈10 d before infestation. The preconditioning process provided us with age-specific aphids with a 3-d age variation.

There were three aphid treatments: 0 aphid, 10 *R. padi* adults, or 10 *D. noxia* adults. The experiment was initiated when plants were at Zadoks stage 13. All plants were caged using polyethylene tubes and randomly arranged in a Conviron growth chamber under the conditions described previously.

Collection of Aphid and Plant Biomass and Chlorosis Evaluation. Aphids and excised wheat plants were collected and weighed on the third, sixth, ninth, and 12th d after initial aphid infestation. In each sampling date, three Conetainers (two plants per Conetainer) of each genotype (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*) under each aphid treatment (control, *D. noxia*-infested, and *R. padi*-infested) were randomly collected. For each Conetainer, aphids were brushed off from the two plants and weighed. Leaf chlorosis of the two plants was quantified using a nine-point rating scale described by Webster (1990), where 1, plants look healthy and have scattered yellow spots; 2, isolated chlorotic spots obvious; 3, chlorosis ≤15% of total leaf area, chlorotic lesions coalesced; 4, chlorosis >15% but ≤25% of total leaf area, leaf streaks occur; 5, chlorosis >25% but ≤40% of total leaf area, obvious streaks; 6, chlorosis >40%, but ≤55% of total leaf area; 7, chlorosis >70%, but ≤85% of total leaf area; 9, plant looks dead or beyond recovery.

After evaluation of leaf chlorosis, the two wheat plants from each Conetainer were excised and weighed. A subsample (0.3 g) was then randomly selected for pigment assay, whereas the rest of the leave sample was analyzed for chlorophyll degradation enzyme activities. Leaf samples were stored in a −20°C freezer before analysis of pigment concentration and enzyme activity.

Photosynthetic Pigment Measurement. The leaf subsample (0.3 g) was ground with liquid nitrogen in a mortar and pestle under low-light conditions. Ace-

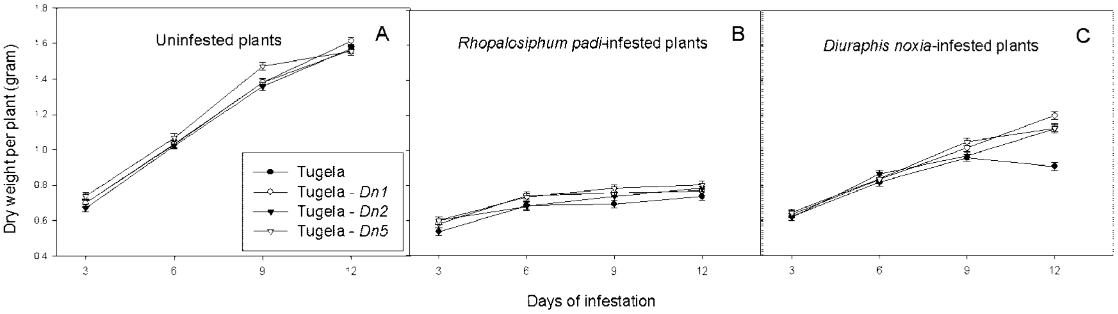

Fig. 1. Temporal changes of plant biomass. (A) Uninfested Tugela plants. (B) *R. padi*-infested Tugela plants. (C) *D. noxia*-infested Tugela plants. Each data point represents the mean ($n = 18$) on each sampling date. Error bar indicates the standard error of the mean.

tone (3 ml of 80%) was added to extract photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and total carotenoids). Approximately 1.5 ml of the mixture was aspirated by polyethylene pipette (Fisher, Pittsburgh, PA) and centrifuged at 6,000 × *g* for 10 min to remove insoluble plant tissues. The final supernatant was diluted with 80% acetone and absorbance readings at 663 nm were adjusted between 0.1 and 1.5 to ensure accuracy. The absorbance of pigment extracts were measured using a spectrophotometer (model Genesys 5, Spectronic Instruments, Rochester, NY), respectively, at wavelength of 470, 646, and 663 nm. Concentration of the three types of pigments was obtained following the equation described by Bertrand and Schoefs (1997):

$$C_a = 12.21\ A_{663} - 2.81\ A_{646}$$

$$C_b = 20.13\ A_{646} - 5.03\ A_{663}$$

$$C_c = (1000\ A_{470} - 3.27\ C_a - 104\ C_b)/198$$

where C_a, C_b, and C_c are the concentrations in micrograms per milliliter of chlorophyll *a*, chlorophyll *b*, and total carotenoids, respectively. A_x represents the absorbance at X nm. Final pigment concentrations were determined in microgram per gram of fresh wheat leaf tissue (microgram per gram). Thus, $C_{a,\ b,\ c\ (\text{final})} = (C_{a,\ b,\ c} \times r \times v)/w$ (r is the dilute ratio of pigment measurement, v and w are the volume of 80% acetone to extract the pigments and the weight of sample plant leaves, respectively). C_a/C_b and C_{a+b}/C_c also were calculated to determine the photosynthetic efficiency.

Experimental Design and Data Analysis. The experiment was a split-split plot design and replicated six times. Four sampling dates (3rd, 6th, 9th, and 12th) were the main plots within each trial. Three aphid treatments (control, *D. noxia*, and *R. padi*) were the subplots within each main plot (sampling date), and the four wheat lines (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*) were the sub-subplots within each subplot (aphid treatment). Six plants were used for each treatment on each sampling date; therefore, 36 plants in total were used per treatment per sampling date. Data were analyzed using the PROC GLM procedure of the SAS software followed by TEST statements to ensure correct error terms were used in assessing main effects of experimental factors (Cochran and Cox 1957, SAS Institute 1989). The means were separated using the Fisher's least significant difference (LSD) test ($\alpha = 0.05$).

Results

Plant Biomass. Plant biomass was significantly affected by the wheat line × aphid treatment × sampling date interaction ($F = 2.41$; df = 18, 90; $P = 0.0034$). Temporal changes of plant biomass were therefore analyzed within each aphid treatment.

Plant biomass of *R. padi*-infested and uninfested plants was not affected by the wheat line × sampling date interaction (*R. padi*-infested: $F = 0.81$; df = 9, 45; $P = 0.6116$; uninfested: $F = 0.67$; df = 9, 45; $P = 0.7353$) and not significantly different among wheat lines (*R. padi*-infested: $F = 2.12$; df = 3, 15; $P = 0.1409$; uninfested: $F = 1.56$; df = 3, 15; $P = 0.2393$) but different among sampling dates (*R. padi*-infested: $F = 15.95$; df = 3, 15; $P < 0.0001$; uninfested: $F = 723.2$; df = 3, 15; $P < 0.0001$). A significant wheat line × sampling date interaction was observed on the biomass from *D. noxia*-infested plants ($F = 5.20$; df = 9, 45; $P < 0.0001$), which indicated different growth rates among the wheat lines. Biomass of *D. noxia*-infested plants were significantly different among wheat lines ($F = 6.19$; df = 3, 15; $P < 0.0001$) and sampling dates ($F = 86.22$; df = 3, 15; $P < 0.0001$).

Biomass of uninfested plants was higher than aphid-infested plants (Fig. 1). Although injury symptoms (chlorosis and leaf rolling) occurred only on *D. noxia*-infested plants, plant biomass of all *D. noxia*-infested wheat lines were greater than *R. padi*-infested plants on all sampling dates (Fig. 1). Tugela isolines, previously reported to be resistant to *D. noxia* (du Toit 1987, 1989), were not resistant to *R. padi*. We found when infested by *D. noxia*, all Tugela *Dn* plants sustained biomass increase throughout the infestation period; however, biomass of Tugela decreased on day 12 (Fig. 1C). This finding confirmed that Tugela was susceptible to *D. noxia* infestation, whereas Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5* were resistant. No significant difference in plant biomass was detected

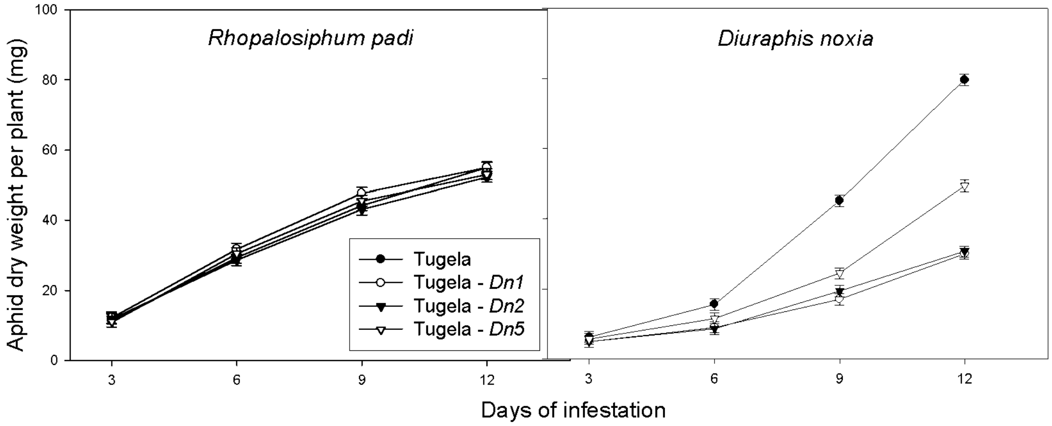

Fig. 2. Temporal changes of aphid biomass. (A) Biomass of *D. noxia.* (B) Biomass of *R. padi.* Each data point represents the mean (n = 18) on each sampling date. Error bar indicates the standard error of the mean.

among *R. padi*-infested or uninfested Tugela wheat lines (Fig. 1A and B).

Aphid Biomass. Biomass of aphids collected from Tugela wheat lines was significantly affected by the wheat line × aphid treatment × sampling date interaction (F = 4.39; df = 9, 45; P = 0.0004). Temporal developments of *R. padi* and *D. noxia* biomass were therefore analyzed.

R. padi biomass was not affected by the interaction between sampling date and wheat line (F = 0.63; df = 9, 45; P = 0.7686). No significant difference was detected in biomass of *R. padi* collected from different wheat lines (F = 2.51; df = 3, 15; P = 0.0983) (Fig. 2A). Biomass of *R. padi* collected on different sampling dates was significantly different (F = 178.82; df = 3, 15; $P < 0.0001$).

Biomass of *D. noxia* was significantly affected by the interaction between sampling date and wheat line (F = 10.05; df = 9, 45; $P < 0.0001$) (Fig. 2B), which indicated *D. noxia* growth rate was differentially impacted by the varying resistance among wheat lines. Biomass of *D. noxia* was significantly different among wheat lines (F = 202.09; df = 3, 15; $P < 0.0001$). The biomass of *D. noxia* collected from Tugela plant was higher than from the other Tugela *Dn* lines (Fig. 2B). Biomass of *D. noxia* collected from Tugela-*Dn5* was higher than Tugela-*Dn1* and Tugela-*Dn2*. No significant difference in aphid biomass was detected between Tugela-*Dn1* and Tugela-*Dn2*.

Chlorosis Rating. Because *R. padi* was a nonchlorosis-eliciting species and did not cause any visual injury symptoms after its colonization on wheat, chlorosis rating was only conducted on *D. noxia*-infested plants. Chlorosis ratings of *D. noxia*-infested plants were affected by the wheat line × sampling date interaction (F = 3.63; df = 9, 45; P = 0.0018), which indicated varying rates of chlorosis among *D. noxia*-infested wheat lines. Significant differences in chlorosis were detected among wheat lines (F = 75.11; df = 3, 15; $P < 0.0001$) and sampling dates (F = 11.12; df = 3, 15; P = 0.0004). Chlorosis was highest in Tugela and lowest in Tugela-*Dn2* plants, whereas chlorosis was higher in Tugela-*Dn1* compared with Tugela-*Dn2* plants (Fig. 3). We also noticed that chlorotic injury increased through time on *D. noxia*-infested Tugela plants. Tugela *Dn* lines showed, however, less chlorosis on day 12 than Tugela plants (Fig. 3). This indicated that Tugela *Dn* plants were more resistant to the injury caused by *D. noxia* feeding.

Chlorophyll *a* Concentration. Chlorophyll *a* concentration was not affected by wheat line × aphid treatment × sampling date interaction (F = 1.06; df = 18, 88; P = 0.4051). None of the two-way interactions were significantly different (P values >0.05). Chlorophyll *a* concentrations were significantly different among aphid treatments (F = 7.83; df = 2, 10; P = 0.0090) and wheat lines (F = 4.04; df = 3, 15; P = 0.0272) but not among sampling dates (F = 0.28; df = 3, 15; P = 0.8405). Injury symptoms (e.g., chlorosis and leaf rolling) were observed on *D. noxia*-infested plants but not on *R. padi*-infested and uninfested plants.

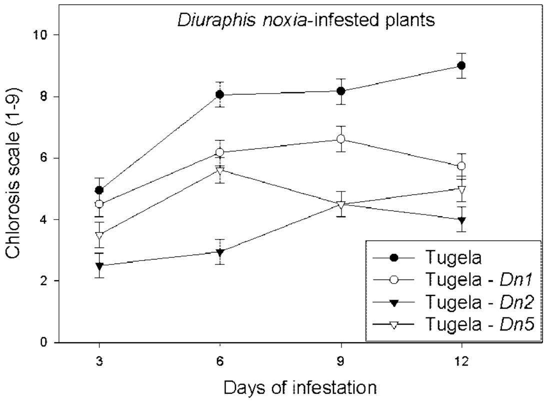

Fig. 3. Temporal chlorosis ratings in *D. noxia*-infested Tugela wheat lines. Each data point represents the mean (n = 18) of chlorosis rating on each sampling date (n = 3). Error bar indicates the standard error of the mean.

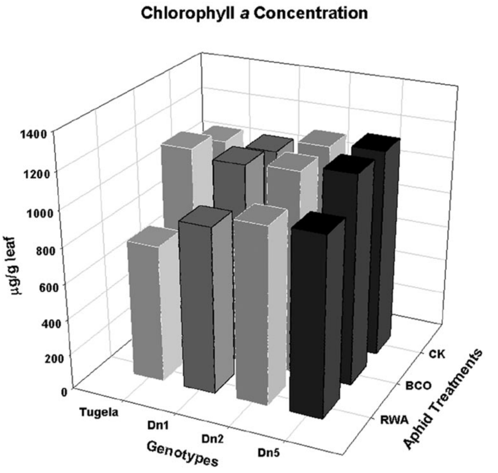

Fig. 4. Chlorophyll *a* concentration in Tugela wheat lines (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*). RWA, *D. noxia*; BCO, *R. padi*; CK, control.

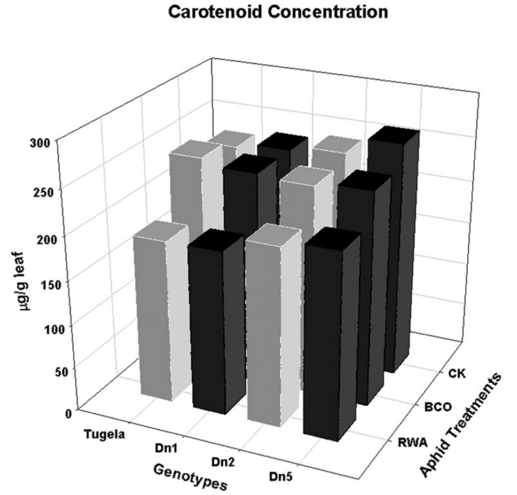

Fig. 6. Carotenoid concentration in Tugela wheat lines (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*). RWA, *D. noxia*; BCO, *R. padi*; CK, control.

Chlorophyll *a* concentration was not significantly different between *R. padi*-infested and uninfested plants, but it was lower in *D. noxia*-infested wheat lines (Fig. 4).

Chlorophyll *b* Concentration. Chlorophyll *b* concentration was significantly affected by the wheat line × aphid treatment × sampling date interaction ($F = 2.06$; df = 18, 88; $P = 0.0141$). Analysis of chlorophyll *b* concentration was therefore conducted within each aphid treatment.

Similar to the analysis of chlorophyll *a* content, chlorophyll *b* was significantly lower in *D. noxia*-infested compared with *R. padi*-infested and the uninfested plants (Fig. 5A–C). Neither wheat line × sampling date interaction nor main effects on chlorophyll *b* concentration was observed in *R. padi*-infested and uninfested plants (P values >0.05). Although no different chlorophyll *b* concentration observed on day 3 among *D. noxia*-infested wheat lines (Fig. 5C), significantly lower chlorophyll *b* concentration was found in Tugela on days 6, 9, and 12. *D. noxia*-elicited injury symptoms also were obvious on Tugela-*Dn1* plants. Additionally, chlorophyll *b* concentration was lower in Tugela-*Dn1* on days 6 and 12 compared with Tugela-*Dn2* and Tugela-*Dn5*. There were no differences of chlorophyll *b* concentrations between *R. padi*-infested and the uninfested Tugela wheat lines.

Carotenoid Concentration. Carotenoid concentration was not affected by the wheat line × aphid treatment × sampling date interaction ($F = 1.28$; df = 18, 88; $P = 0.2235$). None of the two-way interactions affected carotenoid concentration (P values >0.05). Carotenoid concentrations were significantly different among aphid treatments ($F = 7.90$; df = 2, 10; $P = 0.0088$) but not wheat lines ($F = 2.6$; df = 3, 15; $P = 0.0906$) or sampling dates ($F = 1.06$; df = 3, 15; $P = 0.3965$).

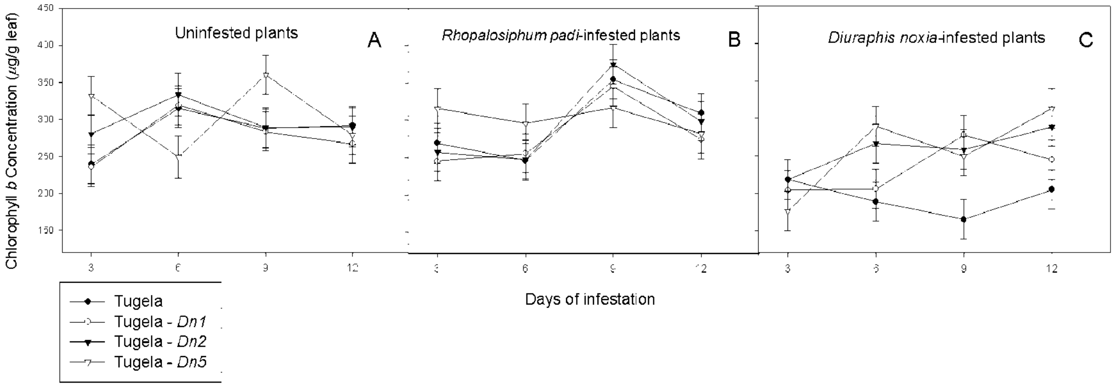

Fig. 5. Temporal changes of chlorophyll *b* concentration (micrograms per gram of leaf). (A) Uninfested Tugela plants. (B) *R. padi*-infested Tugela plants. (C) *D. noxia*-infested Tugela plants ($n = 6$). Each data point represents the mean ($n = 6$) on each sampling date. Error bar indicates the standard error of the mean.

Carotenoid concentration was lower in *D. noxia*-infested wheat lines compared with *R. padi*-infested and the uninfested plants (Fig. 6). There were no significant differences of carotenoid concentrations between *R. padi*-infested plants and the uninfested plants. Unlike the analysis of chlorophylls *a* and *b*, carotenoids were not significantly different among the Tugela wheat lines when infested by *D. noxia.*

Chlorophyll *a*/*b* Ratio. The chlorophyll *a*/*b* ratio in Tugela wheat lines was not significantly affected by the wheat line × aphid treatment × sampling date interaction ($F = 1.01$; df = 18, 88; $P = 0.4564$). None of the two-way interactions had significant impact on the chlorophyll *a*/*b* ratio (P values >0.05). Chlorophyll *a*/*b* ratio was not significantly different among aphid treatments ($F = 0.66$; df = 2, 10; $P = 0.5391$), wheat lines ($F = 0.21$; df = 3, 15; $P = 0.8883$), or sampling dates ($F = 1.29$; df = 3, 15; $P = 0.3141$).

Chlorophyll/Carotenoid Ratio. The chlorophyll/carotenoid ratio was not affected by the wheat line × aphid treatment × sampling date interaction ($F = 0.89$; df = 18, 88; $P = 0.5908$) or by any of the two-way interactions (P values >0.05). Chlorophyll/carotenoid ratios did not differ among sampling dates, wheat lines, or aphid treatments (P values >0.05).

Discussion

Both plant and aphid biomass analysis supported the previous reports by du Toit (1987, 1989) that Tugela is susceptible to *D. noxia* infestation but Tugela *Dn* plants are resistant. When infested by *D. noxia,* Tugela plants showed severe chlorotic symptoms and decreased plant biomass on day 12 compared with the Tugela *Dn* plants (Figs. 1C and 3). Although chlorotic symptoms were only observed on *D. noxia*-infested wheat plants, plant biomass of *R. padi*-infested wheat was lower than *D. noxia*-infested plants (Fig. 1B and C). Therefore, Tugela wheat lines were resistant to *D. noxia* but not to *R. paid* infestation. We found that *D. noxia* biomass from Tugela-*Dn5* (moderately antibiosis) was higher compared with Tugela-*Dn1* (antibiosis) and Tugela-*Dn2* (tolerance) (Fig. 2B). Budak et al. (1999) observed similar results when compared the biomass of *D. noxia* collected from different 'Betta' wheat lines with the same *Dn* genes. They concluded Betta-*Dn5* did not show the same level of resistance to *D. noxia* as the donor line PI 294994 and suggested that resistance inherited from PI 294994 might be controlled by more than one gene. Zhang et al. (1998) in their genetic study of PI 294994 indicated that there might be four subaccessions in PI 294994 and that the progeny may inherit only one or two genes. It is, therefore, possible that Tugela-*Dn5* did not fully inherit the resistance genes from PI 294994. Biomass of *D. noxia* collected from Tugela-*Dn1* was lower compared with Tugela and Tugela-*Dn5* (Fig. 2B). The results support that Tugela-*Dn1* is antibiotic and has negative impact on the biology of *D. noxia.* It is worth noting that we observed less chlorosis on Tugela-*Dn2* plants after *D. noxia* infestation and the biomass of *D. noxia* collected from Tugela-*Dn2* was similar to that from Tugela-*Dn1*, but less than Tugela and Tugela-*Dn5.* Tugela-*Dn2* seemed to be both tolerant and antibiotic to *D. noxia* feeding. Similar results were reported by Haile et al. (1999) and Heng-Moss et al. (2003) when *D. noxia* fed on Betta-*Dn2* (tolerance) wheat.

Chlorophylls *a* and *b* are the primary pigments in plants to harvest light energy for photosynthesis. Because *D. noxia* feeding causes chlorosis in plants, their feeding could potentially affect plant photosynthetic capacity. Chlorophyll concentrations in *D. noxia*-infested Tugela-*Dn2* and Tugela-*Dn5* wheat were significantly higher than Tugela and Tugela-*Dn1* plants. Tugela-*Dn2* and *Dn5* plants were better able to sustain *D. noxia* damage and therefore maintain chlorophyll concentrations and photosynthetic potential than Tuegla-*Dn1* and Tugela (Figs. 4 and 5C). Ni et al. (2001, 2002) detected significantly higher Mg-dechelatase activities in *D. noxia*-infested wheat leaves compared with those of *R. padi*-infested and the uninfested plants. In the enzymatic analysis part of the experiment, we detected higher chlorophyllase activities in asymptomatic *R. padi*infested plants and higher Mg-dechelatase activities in symptomatic *D. noxia*-infested Tugela plants. When infested by *D. noxia,* Tugela showed increased levels of plant chlorosis and lower Mg-dechelatase activity than the isolines with *D. noxia*-resistant genes. Chlorophyll loss in *D. noxia*-infested wheat plants is therefore most likely correlated with chlorophyll degradative enzyme activities.

Carotenoids in higher plants are important in photosynthesis and act as accessory light harvesters and harmful quanta quencher (Blankenship 2002). *Diuraphis noxia* feeding caused reduction in carotenoids and thus was detrimental to wheat photosynthesis. Although carotenoid biosynthesis and its correlated enzymes are well characterized (Dangl et al. 2000, Hundle and Hearst 1991, Hundle et al. 1991), there is no clear mechanism of carotenoid degradation. The intermediate steps and nature of the catabolites in the degradation pathway of carotenoids remain largely unclear (Biswal 1995). As a consequence, the biochemical mechanism of carotenoid degradation on Tugela lines caused by *D. noxia* infestation and its correlation with chlorophyll content changes are not clearly understood. What can be delineated is that the lower carotenoid level among the Tugela wheat lines imposed by *D. noxia* feeding would cause higher potential of oxidative damage to plant, which is detrimental to plant physiology (Bi and Felton 1995, Blokhina et al. 2003) and possibly correlated to the loss of chlorophylls (Burd and Burton 1992).

Decrease of the chlorophyll *a*/*b* ratio has been widely reported in the natural process of plant senescence (Wolf 1956, Sanger 1971, Watts and Eley 1981, Bricker and Newman 1982, Adams et al. 1990), whereas the effect of aphid feeding on chlorophyll *a*/*b* ratio differed, ranging from no change to decrease. Burd and Todd (1992) detected a significant reduction of chlorophyll *a*/*b* ratio in *D. noxia*-infested wheat, TAM W-101. Ni et al. (2002) also reported a significantly lower chlorophyll *a*/*b* ratio in chlorotic area when compared with nonchlorotic area of *D. noxia*-infested 'Arapahoe' (susceptible) wheat leaves. However, similar to the results reported by Burd and Elliott (1996) and Heng-Moss et al. (2003), our study showed that the chlorophyll *a*/*b* ratio in Tugela wheat lines was not affected by *D. noxia* feeding, but maintained a 3:1 ratio observed in natural growing plants. This variation in chlorophyll *a*/*b* ratio could be caused by the difference in plant sampling protocol between Ni et al. (2002) and our study. Ni et al. (2002) measured chlorophyll concentrations of wheat leaves after separating chlorotic and nonchlorotic areas. However, we randomly selected a subsample of the whole plant to measure chlorophyll concentrations. Chlorophyll *a* and *b* concentrations in nonchlorotic areas may have masked the variation of chlorophyll *a*/*b* ratio in chlorotic areas of the leaves. The genetic background of wheat (TAM W-101, Arapahoe, and Tugela) also could have contributed to the variation in chlorophyll *a*/*b* ratio.

Acknowledgments

We thank Y. Liu and P. Hofer for assistance in conducting experiments and for maintaining the plant and aphid colonies, and S. Blodgett and P. Bruckner for critical review of the manuscript. We also thank James F. Robison-Cox and John J. Borkowski for advice in data analysis. The research reported here was supported in part by the USDA–NRICGP 99-35302–8628. This is journal series number 2003-25 from the Montana Agricultural Experiment Station.

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Received 14 July 2003; accepted 13 November 2003.